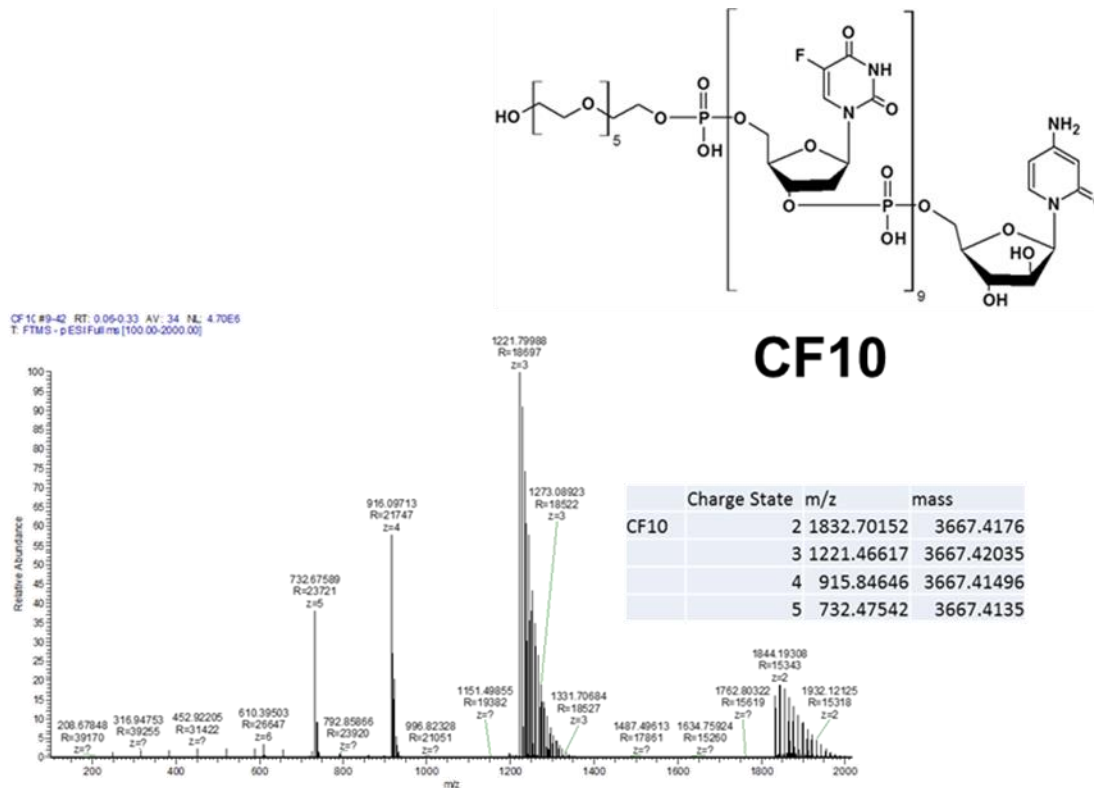


Supplementary Information

Gmeiner et al. "Improved anti-tumor activity of CF10 in pre-clinical CRC models"

A



B

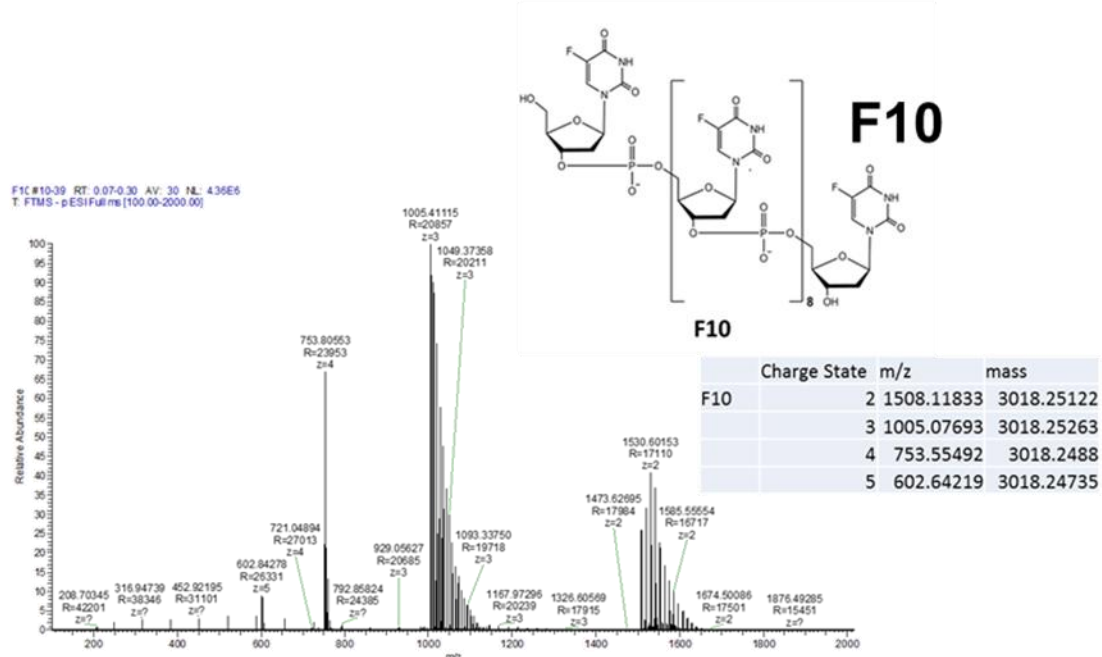


Fig. S1. Mass spectrometry data for (A) CF10 and (B) F10. CF10 was >93% pure based on HPLC analysis and mass spectrometry confirmed chemical identity.

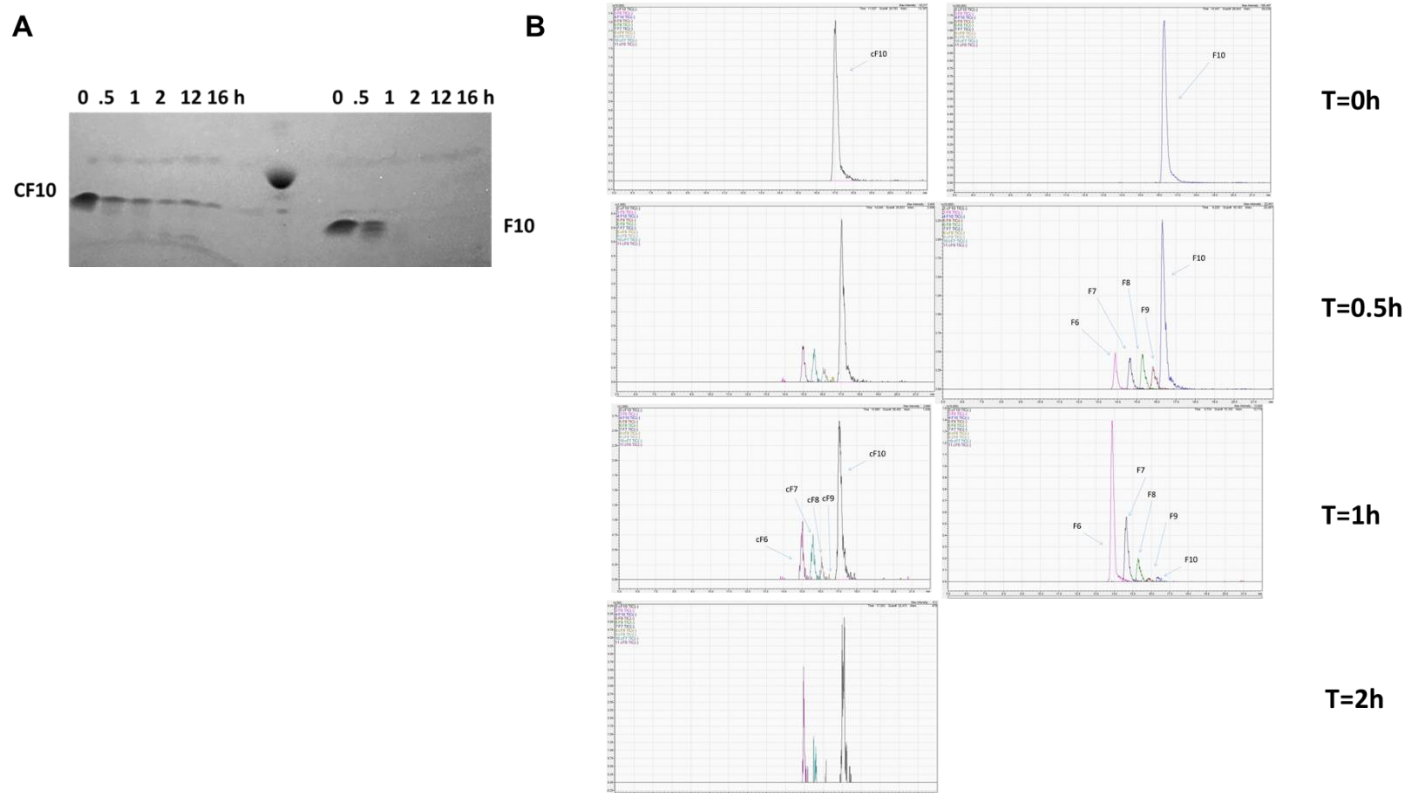


Fig S2. Relative chemical stability of CF10 and F10 to snake venom phosphodiesterase (SVPD) degradation. FP polymers were treated with 0.04 U SVPD for the indicated times and hydrolysis was followed using: (A) 20% PAGE gel and by (B) LC/MS.

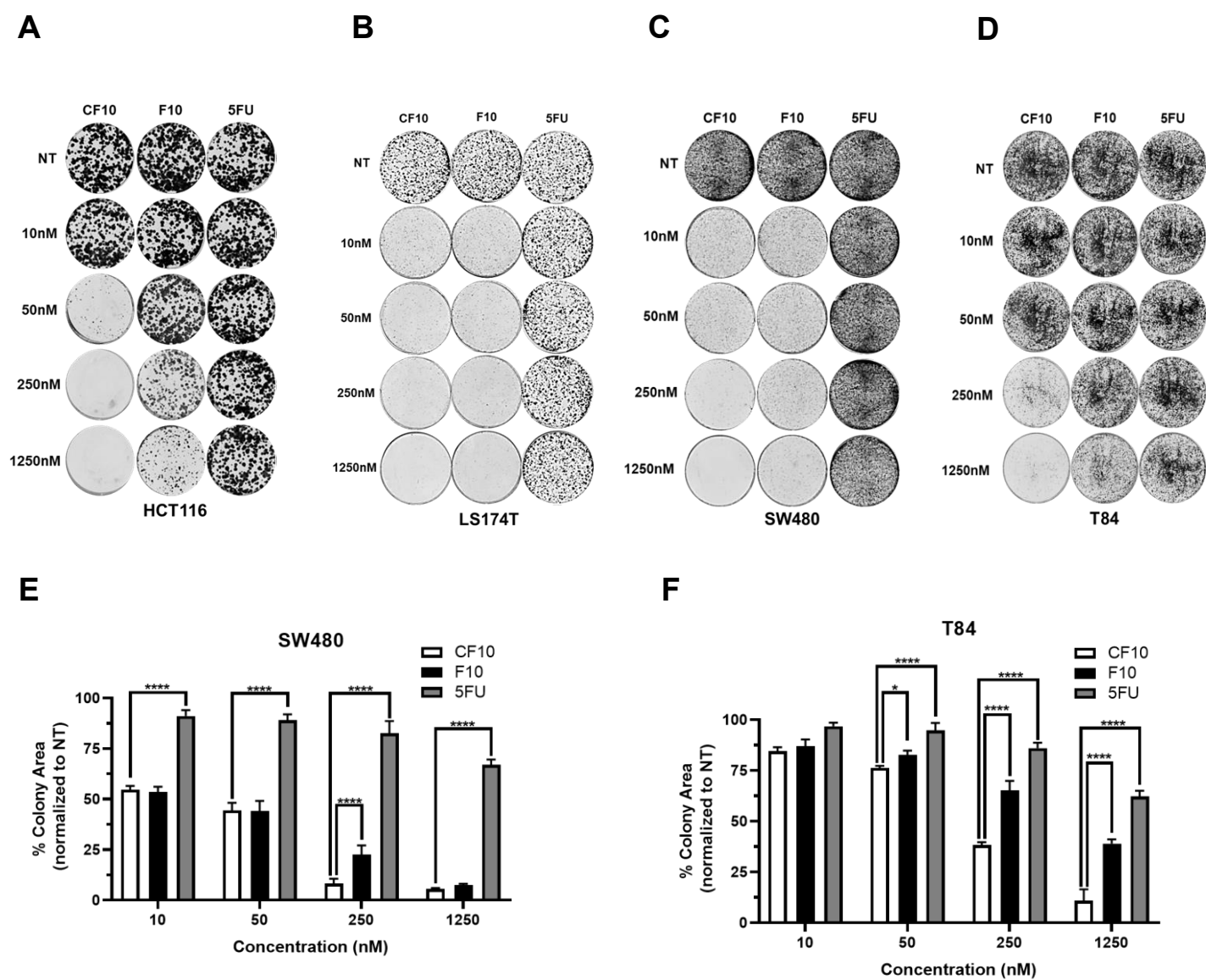


Fig S3. CF10 displays improved inhibition of clonogenic growth relative to F10 and 5-FU in **A**, HCT-116, **B**, LS174T, **C**, SW480, and **D**, T84 CRC cells. E,F Quantification of clonogenic data for **E**, SW480 and **F**, T84 cells. Quantification of clonogenic data for HCT-116 and LS174T is shown in Figure 1 D,E.

IC50 (nM)	CF10	F10	5FU
HCT116	28.90	465.00	3261.00
LS174T	0.15	1.56	4836.00
SW480	7.50	35.26	4243.00
T84	147.80	672.80	4856.00

Table S1. IC50 values from clonogenic assay results in CRC cell lines

Table 1.							
logGI50 Values for Select FPs in CRC Cell Lines (NCI 60 cell line screen)							
	MSI	KRAS	CF10	F10	FdU	TFT	5FU
HCT-116	MSI	G13D	-8.00	-7.96	-6.93	-5.77	-5.39
HT-29	MSS	wt	-8.00	-6.32	-5.55	-4.47	-5.17
Colo205	MSS	wt	-8.00	-6.89	-5.76	-4.50	-5.16
KM12	MSI	wt	ND ¹	-5.45	-5.18	-4.00	-5.06
HCC-2998	MSS	A146T	ND	-7.63	-9.00	-4.95	-5.82
SW620	MSS	G12V	-5.59	-4.30	-5.00	-5.01	-4.55
HCT-15	MSI	G13D	-6.75	-6.51	-5.72	-4.34	-5.25
¹ ND - not determined							

Table S2. Summary of GI50 values from NCI60 cell line screen

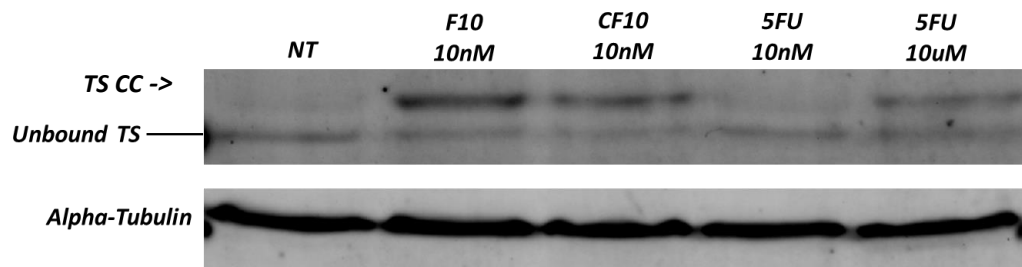


Fig S4. CF10 induces TS ternary complex formation in LS174T cells. Detection and analysis of the ternary complex was similar to that described for HCT-116 cells (Fig. 2A).

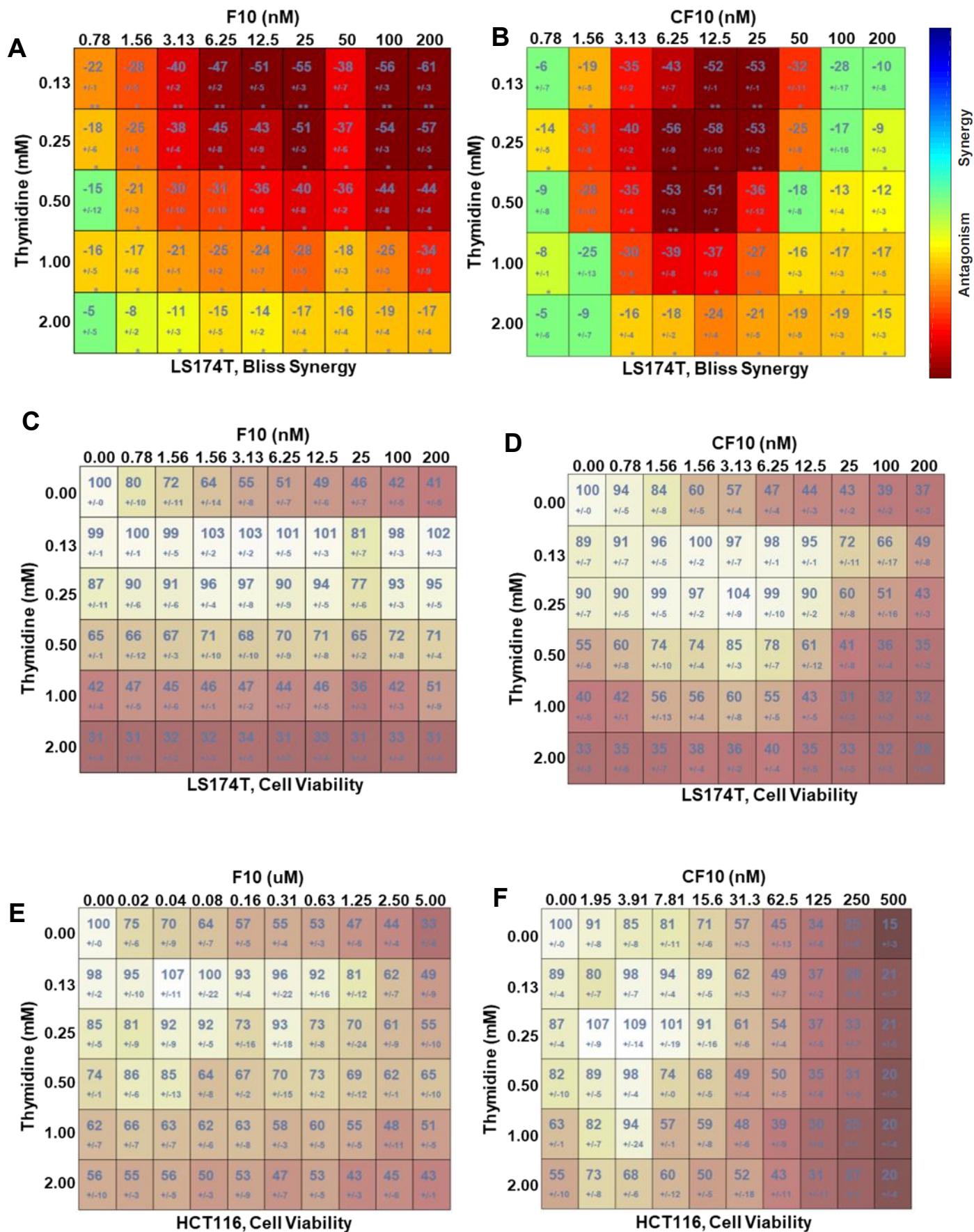


Fig S5. Thymidine antagonism of F10 and CF10 effects on cell viability in LS174T cells. A,B Bliss synergy analysis showing deviation from activity. B,C corresponding cell viability data for A,B. D,E Cell viability data for Bliss synergy data shown in Fig. 1 D,E

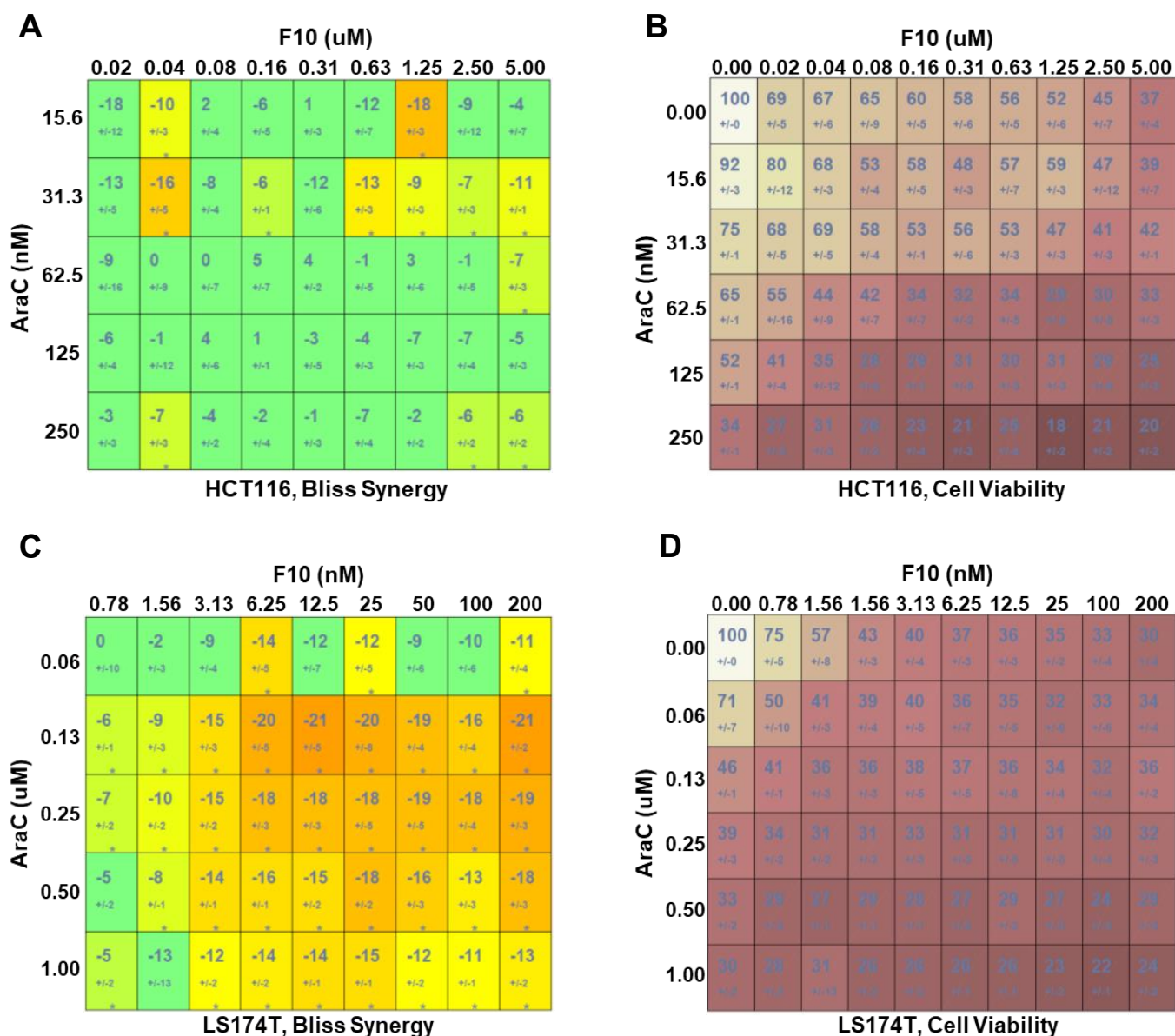
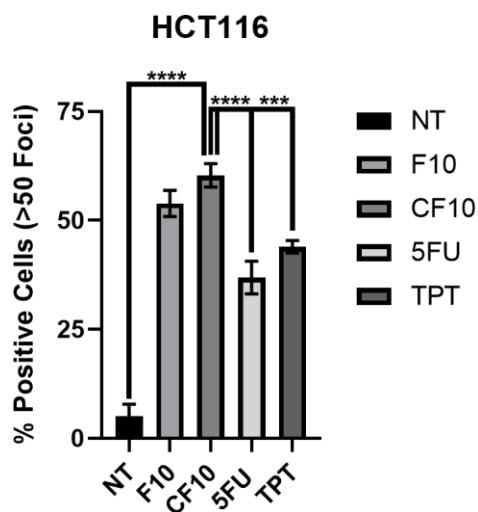
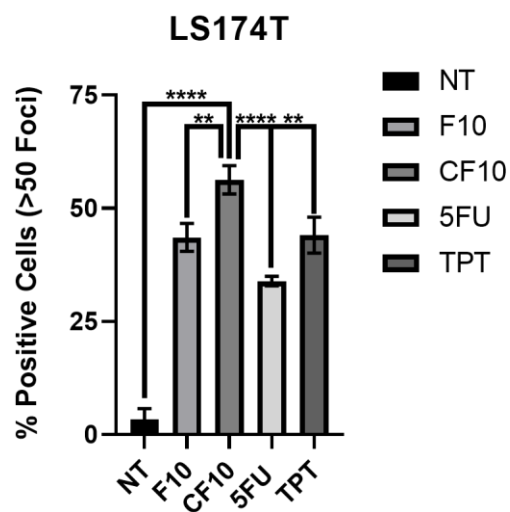


Fig S6. AraC interactions with F10 in **A,B** HCT-116 cells and **C,D** LS174T cells. **A,C** show difference from expected cell viability based on additivity for the indicated concentrations of AraC and F10. **B,C** show viability values. Viability values for AraC in the absence of any F10 are shown in the first column of **B,D**.

Supplementary Table S3. COMPARE analysis results for CF10

Rank	Chemical name	PCC	Target ID
1	5-FUDR	0.642	27640
2	methotrexate	0.635	740
3	AraC	0.596	63878
4	trimetrexate	0.589	352122
5	topotecan	0.582	609699
6	Aphidicolin glycinate	0.574	303812
7	DUP785	0.552	368390
8	Mitomycin C	0.551	26980
9	Cyclocytidine	0.548	145668
10	DON	0.540	7365

A**B****C**

RADAR assay
HCT116, 48 h

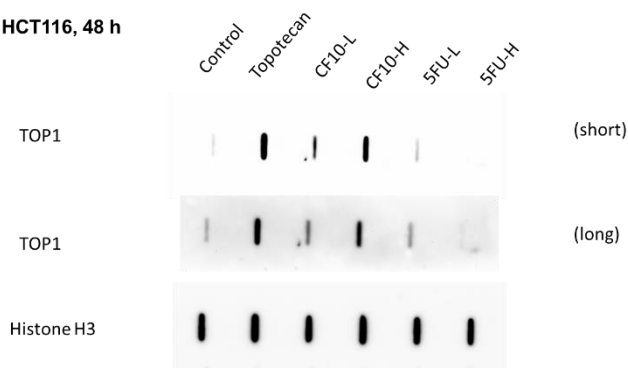


Fig. S7. CF10 induces DNA topoisomerase 1-cleavage complexes (Top1cc) in HCT-116 and LS-174T cells. (A,B) Quantification of Top1cc data from immunofluorescence images shown in Fig 2E. (C) RADAR assay showing detection of Top1cc in HCT-116 cells with both high (10-6M) and low (10-8M) CF10 with minimal detection following treatment with high (10-5M) or low (10-7M) 5-FU. Histone H3 is included as a loading control

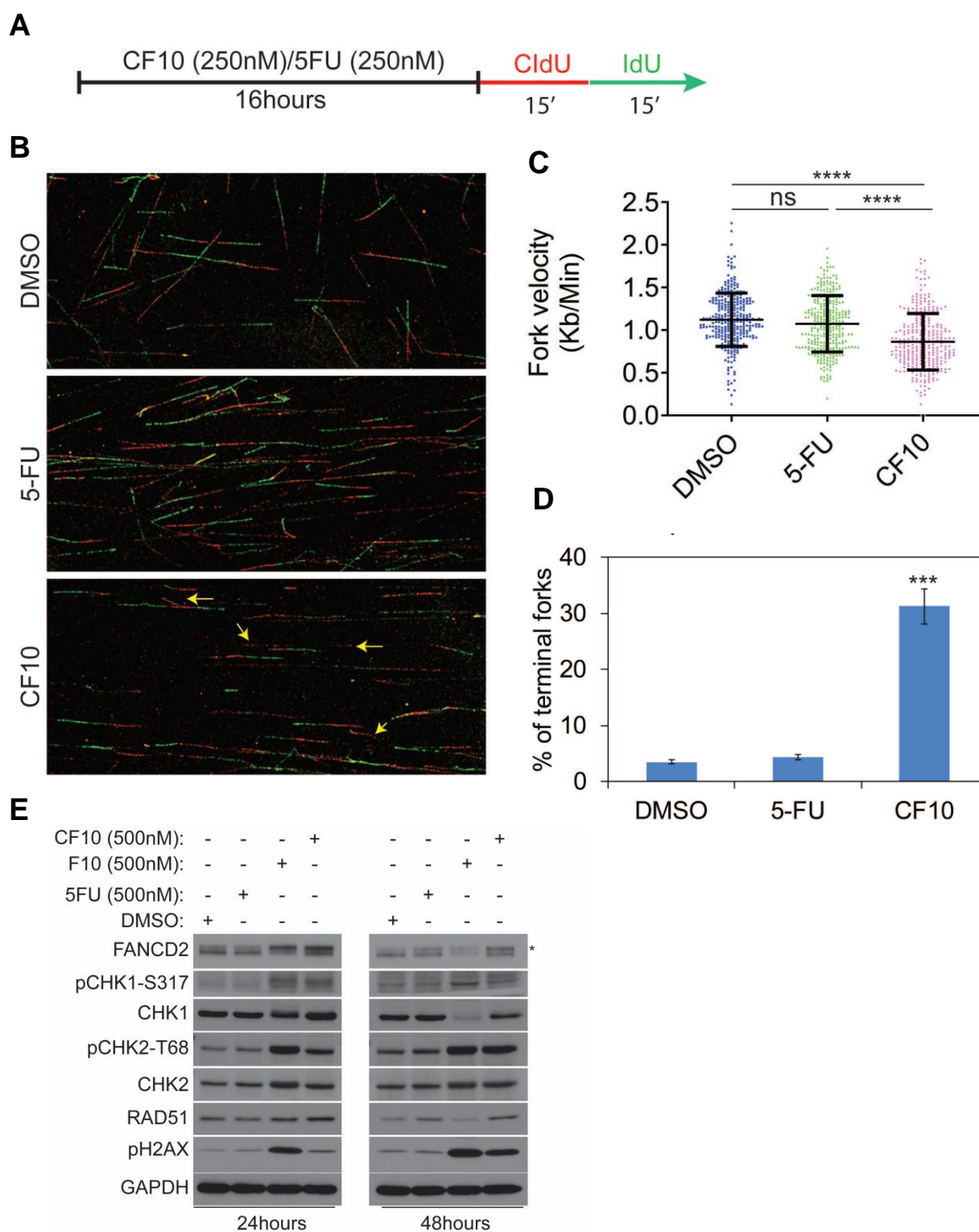


Fig. S8. CF10 causes replication stress and activates the DNA damage response. (A) Scheme used for pulse labeling of DNA for fiber analysis. (B) DNA fiber analysis for CF10 and 5-FU treatment in HCT-116 cells. C,D Quantification of changes in fork velocity and terminal forks following treatment. (E) Western blots from LS174T cells following treatment with indicated concentrations of 5-FU, F10, or CF10 for 24h (left) or 48h (right). FANCD2 levels were increased at 48h CF10 treatment.

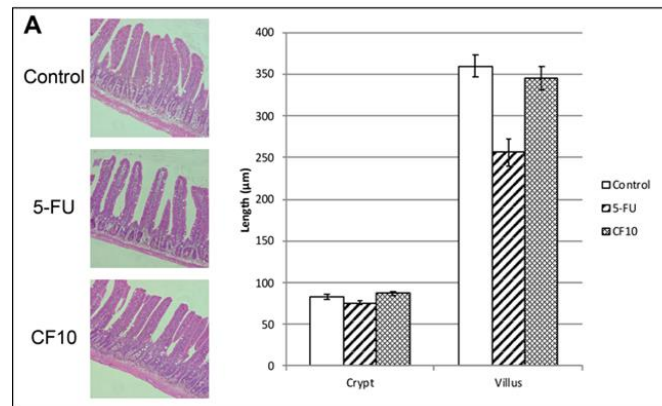


Fig. S9. H&E-stained 4 µm sections of BALB/c mouse small intestine 24 h after a single i.p. injection of PBS (top) 70 mg/kg of 5-FU (middle) or 200 mg/kg of CF10 (bottom). 200 x magnification. Right, quantitative crypt and villus measurements. n= 25 measurements per group. ****p<0.0001 +/- SEM.

Table S4. Summary of blood chemistry parameters following treatment of C57bl/6 mice with 300 mg/kg CF10 or 70 mg/kg 5-FU 2x/wk for 1 week.

	ALT (U/L)	ALT (U/L)	ALT (U/L)		AST (U/L)	AST (U/L)	AST (U/L)		BUN (mg/dL)	BUN (mg/dL)	BUN (mg/dL)		Creatinine (mg/dL)	Creatinine (mg/dL)	Creatinine (mg/dL)
	control	CF10	5-FU		control	CF10	5-FU		control	CF10	5-FU		control	CF10	5-FU
	43	19	113		97	65	887		22	23	25		0.1	0.1	0
	36	44	183		291	127	247		22	18	16		0.1	0.1	0
	29	27	33		206	95	150		22	24	17		0.1	0.1	0
			75				204				17				0
AVG	36	30	101		198	95.66667	372		22	21.66666667	18.75		0.1	0.1	0

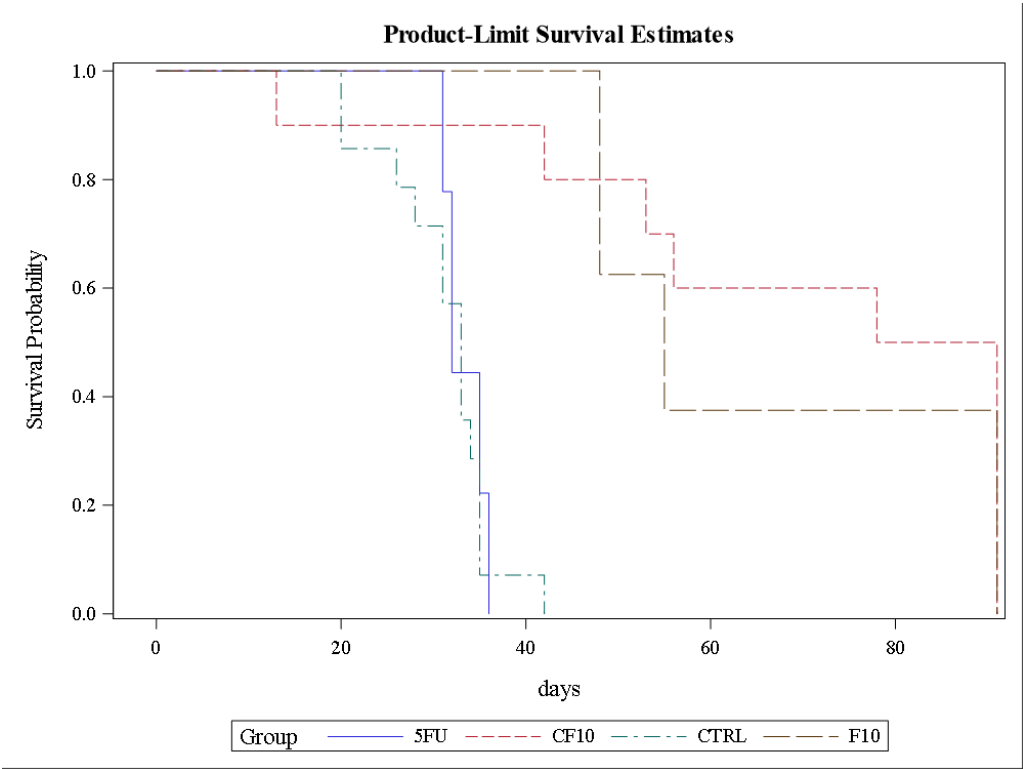


Fig. S10. CF10 trends toward improved survival relative to F10.

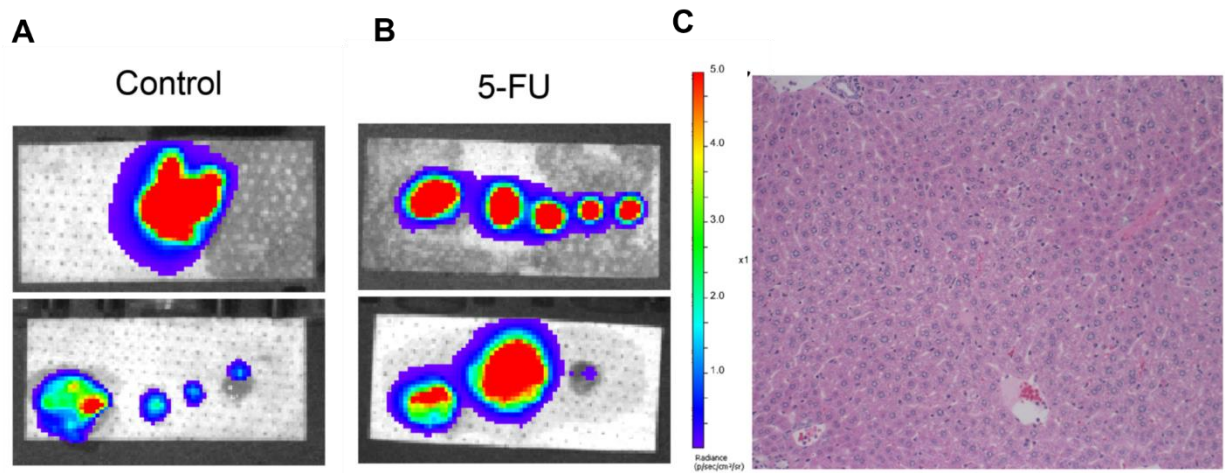


Fig. S11. HCT-116 orthotopic model develops spontaneous metastases to liver that may be inhibited by CF10 treatment. (A,B) IVIS images of resected primary tumor, liver, and suspected metastasis in 2 separate control (A) and 5-FU (B) treated mice. The largest flux in each image is from the primary tumor and the smaller flux are from metastatic and liver tissue. Flux measurements are all within the same scale (right). Tissue sizes are not to scale across different image. (C) H&E stained section of liver from a mouse with HCT-116 orthotopic colon tumor 8 weeks after initiating treatment with CF10. Extensive analysis of liver tissue revealed no signs of metastasis.

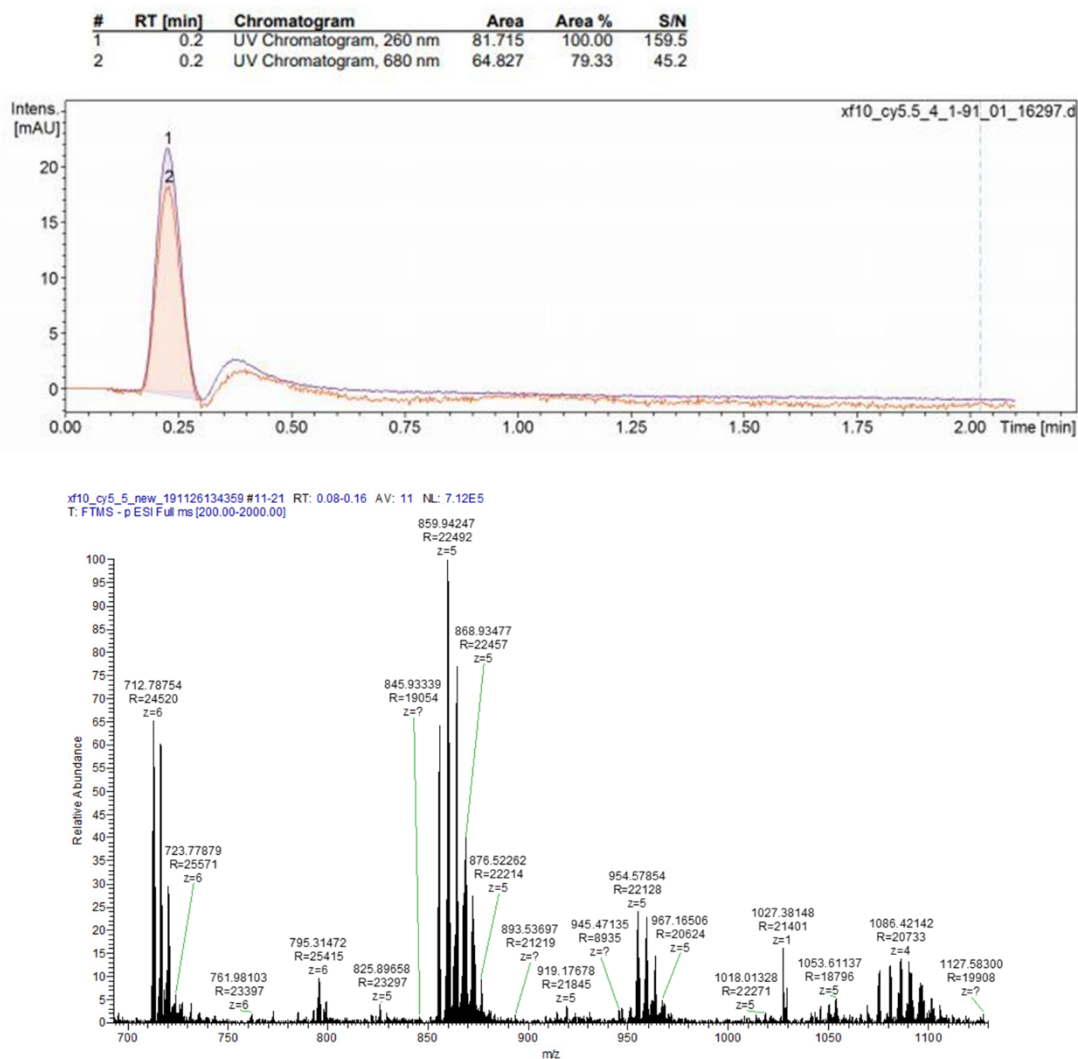


Fig. S12. Analytical data validating formation of the Cy5.5 conjugate of XF10A, a CF10 analog with a 5'-terminal alkyne. (A) Diode array detection of FL_CF10 showing optical absorbance at 680 nm and 260 nm for the conjugate. (B) LTQ Orbitrap XL mass spectrum for FL_CF10.

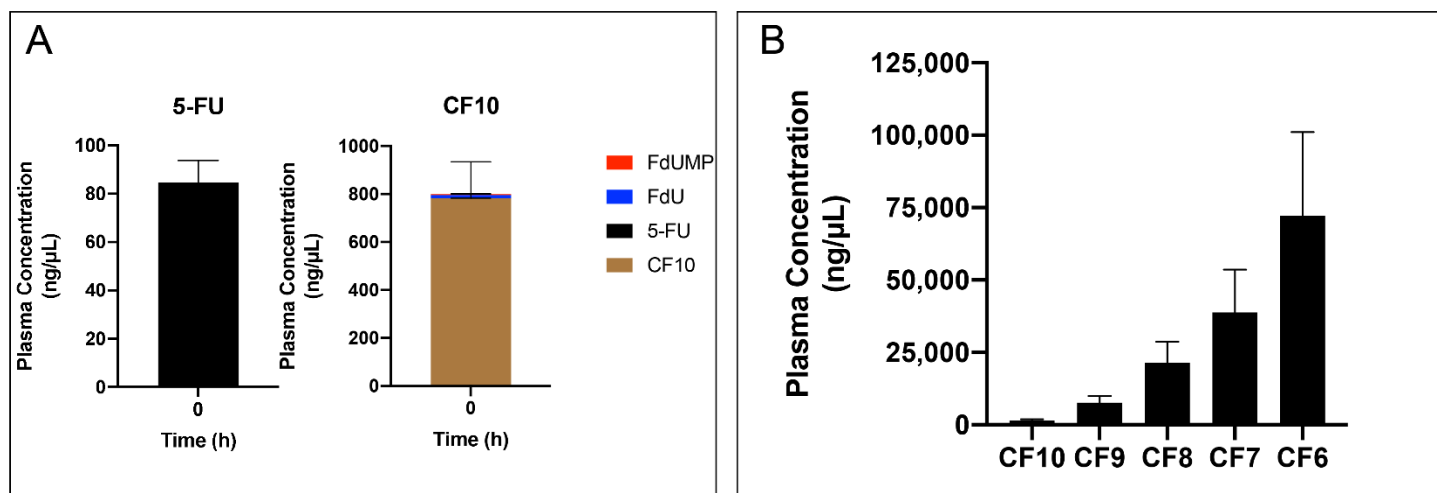


Fig. S13. (A,B) Plasma concentrations of FP metabolites immediately following tail vein injection of 5-FU or CF10. Mice were injected either with 70 mg/kg 5-FU or 300 mg/kg CF10 and blood was rapidly obtained by cardiac puncture under deep isoflurane anesthesia and the indicated metabolites (A) were quantified by LC/MS: FdUMP (red), FdU (blue), 5-FU (black), and CF10 (gold). B, Analysis of the same samples shown in (A) following injection of CF10 at 300 mg/kg but also quantifying shorter multimers resulting from CF10 exonucleolytic degradation.